Reviewed by ESD 10/10/00

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To: BLA #99-1470 File

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Subject: Review of BLA #99-1470

I. INTRODUCTION

SR29142 is recombinant urate oxidase, produced by a genetically modified *Saccharomyces cerevisiae* strain. It is an enzyme which catalyses the oxidation of uric acid to allantoin. The enzyme activity is determined using a test that simulates *in vivo* biological activity. The specific enzyme activity is not less than [redacted] EAU/mg (enzyme activity units) where 1 EAU corresponds to the enzyme quantity which converts [redacted] µmol of uric acid into allantoin per minute in [redacted] buffer at [redacted] ^{0}C.

SR29142 is Sanofi-Synthelabo's internal code number to represent the drug substance. The INN is rasburicase. However, throughout the BLA the code number, SR29142 is used to designate the rasburicase drug substance. The drug substance is a colorless to slightly yellow, clear to slightly opalescent solution stored at [redacted] ^{0}C which contains not less than [redacted] mg of SR29142 per mL of [redacted] buffer. The drug substance is produced at [redacted] located in [redacted] The final drug substance is then shipped to Sanofi-Synthelabo located in [redacted] to be further processed into a lyophilized final dosage form.

The molecule is a tetrameric protein with identical subunits of a molecular mass of [redacted] The tetramer is composed of [redacted] with [redacted] active sites [redacted] The monomer, consisting of a single 301 amino acid polypeptide chain, has no intra or inter-disulfide bridges and is N-terminal acetylated.

SR29 142 is defined by a [redacted] pattern demonstrated by [redacted]

The cDNA coding for SR29142 was cloned from the *Aspergillus flavus* strain, source of [redacted] and inserted into a yeast vector for expression in a strain of *Saccharomyces cerevisiae.*

This is the drug product [redacted]y containing the non-recombinant urate oxidase. A brief comparison between [redacted] and SR29142 is provided in Section 4.1.2.7.

A [redacted] cell bank system (Master Cell Bank and Working Cell Banks) was established. The cell banks are stored [redacted] and are controlled for identity and purity. The genetic stability under production conditions was confirmed. In this study, the production cells were cultivated [redacted] generations beyond the limit of *in-vitro* cell age used for production.

The fermentation process at a target scale of [redacted]L consists of a preculture (initiated from the working cell bank) and a culture stage. SR29142, being [redacted]. The active enzyme is then purified by several chromatographic techniques that take into account different physical and chemical properties of the molecule. In-process testing is performed to ensure drug substance quality and consistency between production runs.

As the process approached commercialization, the drug substance production facility was upgraded and the processes were automated to enhance production efficiency. These improvements involved minor process adjustments and changes to both the facility and equipment.

The drug substance specification is based on a selection of tests for [redacted] They are established based on data for batches of drug substance used in toxicology, clinical and stability studies. The results obtained on [redacted] consecutive clinical batches of drug substance demonstrate process consistency. All methods were validated. A [redacted] months expiry dating at [redacted]°C is proposed for the drug substance.

The BRANDNAME for Injection is a sterile lyophilized powder containing 1.5 mg of SR29 142 with mannitol, alanine and dibasic sodium phosphate. It is reconstituted prior to infusion with a solvent for parenteral use which is a 1 mL sterile solution containing poloxamer 188 and water

for injection. The BRANDNAME for Injection is supplied in a 3 mL, stoppered, clear, glass vial and the solvent for parenteral use in a 2 mL, clear, glass ampoule.

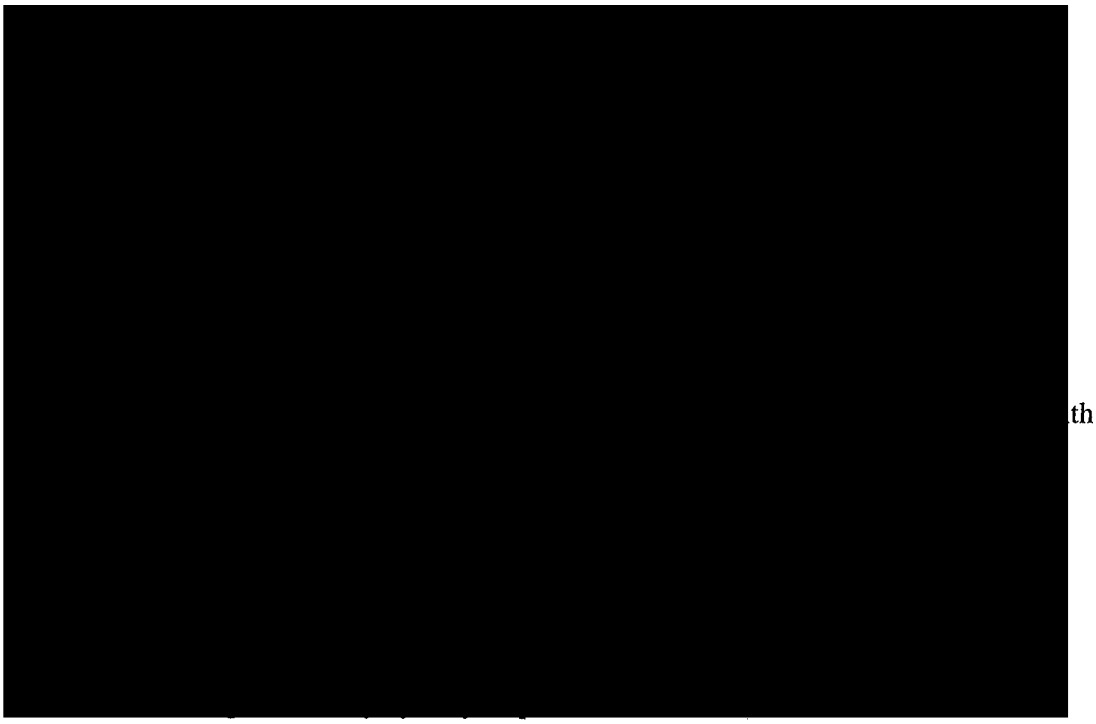
No tradename has been approved as of 5-10-2000. The first three proposed tradenames were rejected by FDA Advertising and Promotional Labeling Branch.

The specifications for BRANDNAME for Injection and for the solvent for parenteral use are based on a selection of tests for identity, purity and impurities, and assay. They are established based on data for batches of BRANDNAME for Injection and solvent for parenteral use used in toxicology, clinical and stability studies. The results obtained on [redacted] consecutive clinical batches of drug product demonstrate process consistency. All methods were validated. The expiry date requested for BRANDNAME for Injection is 36 months at 5 +/-3 ^{0}C . The expiry date requested for the solvent for parenteral use is 48 months stored at +25 ^{0}C or under refrigeration when packaged with the BRANDNAME for Injection.

Studies were performed to demonstrate the stability of the reconstituted solution for 24 hours at [redacted] ^{0}C and compatibility with a variety of infusion devices. After reconstitution the solution is intended for dilution in 5O mL of 0.9 % sodium chloride, prior to infusion.

II. DRUG SUBSTANCE

Urate oxidase is an enzyme of the purine breakdown pathway that catalyses the oxidation of uric acid to allantoin. It is present in many different organisms, but not in higher primates. Urate oxidase from the filamentous fungus, *Aspergillus flavus*, used in human therapy, is one of the best characterized. This enzyme is a tetramer with identical subunits of 34 kDa which are not linked by disulfide bridges. The N-terminal amino acid of the monomer is an N-α-acetylated [redacted] X-ray analysis shows that the tetramer is composed of [redacted]. The [redacted] active sites [redacted] are located [redacted]
To produce a recombinant urate oxidase, the corresponding cDNA from *A. flavus* was cloned and adapted to an expression system involving the yeast *Saccharomyces cerevisiae.* The cloned cDNA codes for a protein of [redacted] amino acids [redacted] The recombinant mate oxidase coded SR29142 was characterized in a series of studies focused on: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These studies were performed on the in house primary reference material

The In House Reference Standard is Batch

A. DESCRIPTION AND CHARACTERIZATION

1. DESCRIPTION

Nomenclature

International non-proprietary name (INN)

Rasburicase

US Pharmacopeia, other pharmacopeia

None

Generic/trivial names

Recommended by IUPAC-IUB:

Urate Oxidase

Other names:

Uricase

Uric acid oxidase

Urico-oxidase

Uratoxidase

Urate: oxygen oxidoreductase

(systematic name assigned by IUPAC-IUB)

EC 1.7.3.3 (Code number assigned by IUPAC-IUB)

Proprietary names/Trademark

Pending

National approved name

USAN application pending for rasburicase

CAS registry number

134774-45 1

Laboratory code

SR29142

Identification number of production strain

[redacted]

Physical form

The drug substance is a colorless to slightly yellow, clear to slightly opalescent solution.

Structural formula

SR29142 is a tetrameric protein with identical subunits; the structure of the monomer, a single 301 amino acid polypeptide chain which is N-terminal acetylated is:

Molecular formula

$C_{1523}\ H_{2383}\ N_{417}\ 0_{462}\ S_7$ (monomer)

Relative molecular mass (Mr)

monomer; calculated from the expected sequence)

Isoelectric point (pI)

(calculated from the expected sequence)

2. CHARACTERIZATION /PROOF OF STRUCTURE

The apparent molecular mass of the monomer and the existence of intermolecular disulfide bridges was evaluated by The precise mass was determined by . The protein (monomer) was

tetrameric structure was established by

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[redacted]

natural urate oxidase from *A. flavus*.

Comparison between SR29142 and [redacted] (Natural Urate Oxidase)

The recombinant urate oxidase SR29142 was developed as a second generation product with respect to [redacted] an enzyme preparation currently marketed in [redacted] The cDNA coding for SR29142 was cloned from the source of [redacted] an *Aspergillus flavus* strain. The amino-acid sequence corresponds to that which was expected.

The application of this production process results in a well characterized and well defined product of high purity. The specific enzyme activity is about [redacted]% higher than that of [redacted] SR29142 presents [redacted] by the same techniques.

With respect to the [redacted] differences are also found for [redacted] These differences are clearly attributed to minor structural modifications occurring during the [redacted] manufacturing process. Nevertheless, the quaternary structures of SR29142 and [redacted] are superimposable.

c. Impurities

Product-Related Impurities

The sponsor characterized and confirmed the identity of minor product-related impurities which were observed in [redacted] sporadically reveals traces of dimer at levels less than [redacted] reveals [redacted] impurities. The sum of product-related impurities ranges from about [redacted]% and these values are essentially due to the presence of related impurity [redacted] and to a minor extent related impurity [redacted] Related impurities [redacted] are below the quantification limit.

Process-related impurities

Of the possible impurities derived from the expression system, the presence of host cell proteins and DNA was investigated in the [redacted] Host-cell proteins (SCP; saccharomyces cerevisiae proteins) are detected at levels less than [redacted] ppm in the [redacted] No DNA was detected using plasmid DNA as a tracer. This finding is in line with the results of the validation of DNA removal (see process validation).

Of possible impurities arising from fermentation, extraction, and purification steps, the presence of [redacted] eventually present, [redacted] was investigated. [redacted] are detected at low levels, always under the quantification limit of the test: less than [redacted] used in the elution buffer of purification step at the concentration of [redacted] thus the low levels found in the drug substance (<5 ppm) demonstrate the efficiency of the two last steps to remove [redacted] The efficiency of these steps is further demonstrated by the removal of [redacted] (detection limit [redacted] ppm). For the [redacted] residual levels found in the crude extract ([redacted] ppm) are further reduced to less than [redacted] ppm in pool [redacted]

Contaminants

The contaminants studied were [redacted] All the batches of drug substance are free of [redacted] as expected from the process conducted so as to minimize the risk of contamination.

B. Manufacturer

The production process described in this section for the batches proposed to be marketed consists of two steps:

- Fermentation and extraction, initiated from the Working Cell Bank and resulting in the crude extract,
- Purification of the crude extract, resulting in [redacted] of the drug substance, SR29142.

The production process was developed to guarantee batch-to-batch consistency and is currently conducted under established procedures following good manufacturing practices. The performance of the process is monitored at each step by in-process controls for which acceptance criteria have been set.

The drug substance is produced in a plant specifically designed so that the premises and equipment meet cGMP and environmental protection requirements for the production of drug substances from genetically modified microorganisms of class 1.

After the clinical batch production, as the development process continued, the production site was upgraded and the process was automated to enhance process reliability. These improvements involved minor process adjustments and changes to both the facility and equipment.

1. Identification

The name, address and reasponsibilities of the drug substance production site are as follows:

Responsibilities:

Starting and raw material testing
In-process and release testing
Drug substance release
Shipping drug substance to the final dosage manufacturing site
Post approval stability studies on commercial batches

2. Floor Diagram

A floor diagram is provided and described on pages 103-107 of volume 1.2.

3. Other Products

There are no other products manufactured at this site.

4. Contamination Precautions

A number of precautions are taken to prevent contamination in the zones in which operations involve handling of the production strain and/or the drug substance (cell bank preparation, fermentation/extraction, and purification steps, and bulk drug substance filling). Building and facility design, including air, fresh and spent process fluid treatment, equipment design, in process controls, and various procedures for conditions of use and cleaning, all contribute to the control of contamination; the major features and procedures related to the building and facilities are briefly described below. Equipment design and basic precautions taken with respect to equipment use, cleaning, and sanitizing or sterilizing are covered in other Sections: 4.1.5.3.2 for cell banks, 4.1.5.3.3 for fermentation and extraction, and 4.1.5.3.4 for purification and filling.

The controls performed to ensure the absence of microbial contamination in cell banks, in the broth during fermentation [redacted] and in the bulk drug substance am detailed in Sections 4.1.5.3.2.2.2, 4.1.6.1.1 and 4.1.6.1.2, respectively.

C. Method(s) of Manufacture

1. Raw Materials And Reagents

All raw materials and reagents used in the manufacture of drug substance are [redacted] with the exception of [redacted]

Note: the [redacted] used in the generation of the master cell bank ([redacted] is derived from bovine non-neural tissue, partially of French origin, produced in 1989.BSE was first identified in 1991 in France. The material was certified (dated [redacted] to be from healthy herds of animals which as of 1/2/93 had no reported cases of BSE. The certificate states that "never the less, the USDA has stated that such an imported material is not to be used for veterinary pharmaceuticals"

[redacted] *This was not used in the preparation of the working cell bank or in fermentation (see page 120-143).*

2. Flow Charts

Flow diagrams for fermentation and extraction were provided on 153-156 and for purification on pages 158-161 of Volume 1.2.

3. Detailed Description

i. Cell Substrate/Host Cell / Expression Vector System

(1). Host Cells

The host cell is a strain of Saccharomyces cerevisiue, named [redacted] which is kept in the [redacted] strain collection under the reference [redacted] is a laboratory strain obtained by classical yeast genetics, including mutagenesis and crosses, from available reference strains [redacted] The affiliation of this strain is summarized in Figure 1 (page 163 of Vol 1.2).

The genotype of [redacted]

The strain [redacted] has mutations in the [redacted] genes, and thus requires a [redacted] in the growth medium. The [redacted] mutation which results in the loss of [redacted] activity renders the strain [redacted] is required for the activation of other [redacted] including [redacted]

The phenotypic properties constitute the criteria used for the controls of the host cell bank and of the production strain (cell seed lot system, production).

The strain [redacted] does not produce endogenous urate oxidase. This point was investigated since S. cerevisiae and A. flavus (Ascomycetes) belong to the same class of

fungi, even though such a possibility was considered unlikely given that S. cerevisiae strains cannot use uric acid as the sole nitrogen source.

This host microorganism is generally recognized as non-pathogenic for man, animals and plants, and has a long history of safe use. It is not known to be a host for viruses posing a risk to higher organisms.

(2). Gene Construct

The strategy for obtaining the segment coding for SR29142 [redacted] coding sequence) was based on the isolation of a cDNA clone from *Aspergillus flavus* (strain [redacted]
The sequence of this cDNA clone was determined, and shown to be identical to the sequence of the gene isolated from A. flavus, with the exception of [redacted] present in the genomic DNA; the size of the open reading frame (ORF) corresponds to a polypeptide sequence of [redacted] (**see pages 165-168 of Vol 1.2** for gene, cDNA and polypeptide sequences) [redacted]
Subsequent work demonstrated that the enzyme produced by the recombinant yeast is [redacted] like the authentic enzyme.
The cDNA was modified to facilitate protein expression in yeast. It was [redacted] the coding sequence was then reconstituted by [redacted] The assembly of the segment coding for SR29142 and the sequence of the [redacted] are shown on **page 169 of Vol 1.2**. The DNA sequence of the [redacted]

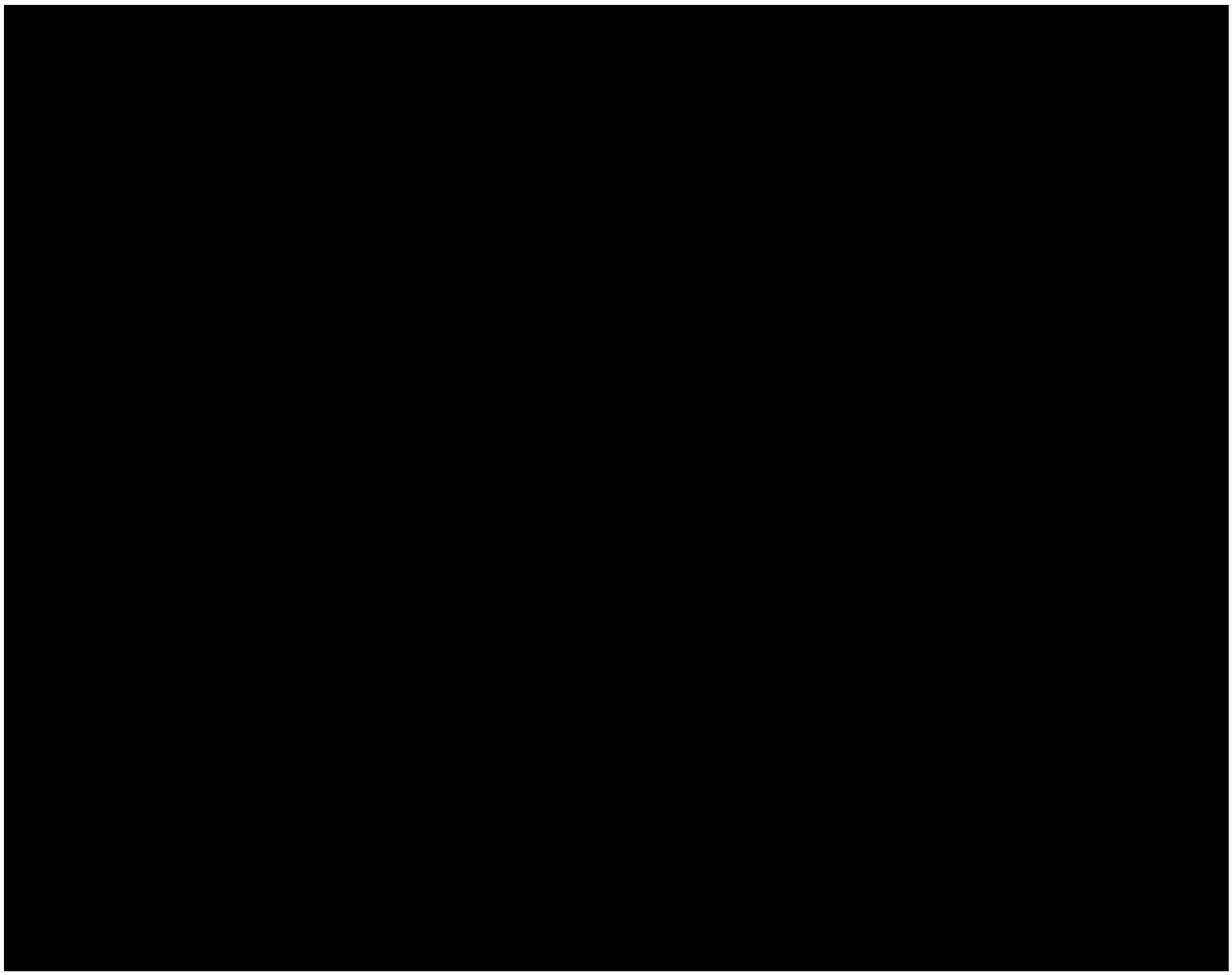
The SR29142 encoding sequence cloned in the expression vector to give the expression construct [redacted] is given on **pages 171-174** with the corresponding polypeptide sequence.

(3). Vector

The expression construct [redacted] is a [redacted] vector which can be [redacted] is derived from the plasmid [redacted] commonly used as a vector for yeast [redacted]

(4). Final Gene Construct

The simplified functional map of the expression construct [redacted] given below indicates the different components. A detailed description of the generation of the final gene construct, as well as a description of components of the final gene construct required for SR29142 gene expression, selection and maintenance in yeast and E. coli are contained on pages 177-192.



The sequence of the expression construct [redacted] prior to transformation was determined experimentally except for the sequences of the terminator [redacted] the first two could be completely deduced from the literature whereas for the third one [redacted], some uncertainties remained with respect to the length of the [redacted] region.

The entire expression construct was later sequenced on a sample of the master cell bank [redacted] and the results are contained on **pages 238-243 of Vol 1.2**.

In conclusion, these results demonstrate that the experimentally determined sequence is consistent with the expected sequence (no sequence variation was observed upon transformation). In particular they allowed determination of the exact sequence and size of the [redacted]-derived fragment including the [redacted] gene.
Plasmid structure was also controlled at different steps [redacted]

The results of the control of the master and two working cell banks are given in Sections 4.1.5.3.2.1.3 and 4.1.5.3.2.2.3, respectively.

(5). Cloning And Establishment Of The Recombinant Cell Lines.

DNA of [redacted] was purified from [redacted] Absence of viable microorganisms and restriction profile were controlled prior to transformation.

[redacted]

[redacted]. This strain was named [redacted]
The [redacted] copy number has been estimated in the production strain as a master cell bank control and [redacted] plasmids/genome were detected.
The [redacted] expression construct is present in the production strain as an [redacted] plasmid in an [redacted]

The transformed cell differs from the host cell only by the presence of the expression construct [redacted] As a result, the transformed cell expresses in addition the following genes:
- the [redacted] encoding gene. The expression of this gene is under control of the [redacted] promoter [redacted] which is maximally induced in the presence of [redacted] and repressed in the presence of [redacted] as shown by the study by [redacted] -transformed cells do not exhibit any detectable urate oxidase activity when grown in [redacted] containing medium. When [redacted] is replaced by [redacted] urate oxidase is produced but only at a very low level, representing less than 0.1 % of total cell proteins. When [redacted] is added to the latter medium, urate oxidase level increases about [redacted] -fold. The synthesis of SR29142 is therefore induced by adding [redacted] into the medium, after complete [redacted] This gene is only weakly expressed in yeast, protein levels estimated at 10^3- 10^4 times less than in E.coli (Roggenkamp et al, 1981). The consequences of such levels on yeast cell biology are considered negligible. Moreover, since yeast is not sensitive to [redacted] nor to [redacted] antibiotics in general, production of active [redacted] does not confer any selective advantage to the production strain.
- the [redacted] gene, the selection marker. This gene restores [redacted] prototrophy in [redacted] producing strain. [redacted] medium is therefore used to select the producing cells. [redacted] gene is also used as a genetic marker to differentiate between [redacted] and production cells .

It should be noted that none of the coding sequences introduced into the host strain via [redacted] confers pathogenic or toxic properties to the production strain ; they are not expected to increase the strain's capacity for proliferation, dissemination, survival or plasmid transfer. The production strain has been attributed class I/group I/containment level 1 by the [redacted] This S. cerevisiae host

vector system would be considered exempt from the NIH guidelines for small scale research and be classed containment level GLSP for large scale research or production.

ii. Cell Seed Lot System

(a). Master Cell Bank (MCB)

The master cell bank (MCB) is composed of [redacted] containing samples of a culture of the SR29142 production strain [redacted] It is referenced as [redacted] The initial size of the MCB constituted in 1991 was [redacted] This supply is consistent with the needs of the long-term commercialization strategy developed for SR29142. The cryotubes containing the MCB are stored in [redacted] The total number of generations is about [redacted] based on cell density estimates.

The following controls for the master cell bank were used for the constitution of the working cell banks. The controls undertaken are of two different natures as described below.

Those of a general nature are:

[redacted]

[redacted] purity

[redacted]

The other controls give information on the characteristics of the production strain. Stability and identity controls for the host cell are:

[redacted]

The stability controls for the expression construct structure are:

[redacted]

The stability controls for the host cell/expression construct association are:

[redacted]

[redacted]

A summary of the results on master cell bank [redacted] can be found on page 236.

The controls of a general nature show that the master cell bank is a pure culture containing about [redacted] viable cells per mL. The identity and stability of the host cell are demonstrated by the findings as expected of a population of unicellular yeasts of [redacted] mating type, without significant c[redacted] and which still require [redacted] for growth [redacted]. The expression construct structure did not undergo any modification as shown by [redacted]

The sequences of the promoter, the coding sequence of the SR29142 gene, the terminator, the [redacted] fragment, [redacted] as illustrated in Figure (4.1.5.3.1.4) 3 found in the section on the final gene construct, and the sequence of the yeast [redacted] plasmid derived subfragment of [redacted] the same figure) are identical to the expected sequences. The length of the [redacted] tails were determined to be:

[redacted] for the SR29142 cDNA [redacted]

[redacted] for the LEU2-d gene [redacted]

These latter results permit to determine the exact sequence and size of the [redacted] derived fragment including the [redacted] gene with its [redacted] However, the [redacted] gene presents [redacted] mismatches at position [redacted] as compared with the sequences available in the gene bank (Accession no. [redacted] They are likely due to an [redacted] since [redacted] and the other [redacted]

Therefore, the experimental sequence that was determined agrees with the expected sequence, except for an [redacted]

The stability of the association of the host cell and expression construct is shown by the high percentage of cells retaining their plasmid [redacted] with a high copy number estimated at [redacted] plasmids per genome. Furthermore, the capacity of the cells to produce urate oxidase in a fermentor was confirmed. These master cell bank results are considered acceptable for the production of SR29142.

(b). Working Cell Bank

Each working cell bank (WCB) is prepared from [redacted] of the master cell bank. The WCB on which was based the production of SR29142 for toxicology and clinical studies is [redacted] prepared on [redacted] That used for the production of batches by the commercial process is [redacted] prepared on [redacted]

The cryotubes of the WCB are stored under the same conditions as the MCB, that is in [redacted] The stability of the two WCBs cited above stored for [redacted] was investigated. The results comply with acceptance criteria and demonstrate the good viability and stability of the WCB over this period. One WCB is expected to last up to [redacted] years. The number of generations is estimated at [redacted] the calculation is based on the initial celldensity used for preparing the WCB ([redacted] cells/ml) and the final cell density ([redacted] cells/ml).

The routine controls for the WCB are the same as those undertaken for the master cell bank except for the addition of [redacted] to ensure that each fermentation is inoculated with the same quantity of cells. Given that other tests performed provide the same type of information, the [redacted]

Each control is described in the following section by the method and the acceptance criterion on pages 247-253. The acceptance criteria of the working cell bank are derived on the results of the master cell bank. The data are presented on page 252.

(c). End of Production Cells (EPC)

[redacted]

[redacted]

iii. Cell Growth and Harvesting

The fermentation process was developed to produce consistently a high level of the drug substance SR29142, particularly by ensuring strain stability and culture purity. The fermentation is carried out in two steps:

- preculture (initiated from the working cell bank)
- culture

The culture consists of [redacted] differing by the nature and method of [redacted] The process was developed taking advantage of the ability of yeast to [redacted] as follows:

[redacted]